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[RAPID COMMUNICATION]

Nucleotide Sequence of the Proton ATPase Beta-Subunit Homologue of the Sea Urchin *Hemicentrotus pulcherrimus*[1]

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ABSTRACT—A cDNA with 2.3 kb encoding F_1-F_0 ATP synthase (proton ATPase) beta-subunit homologue was isolated from a testis cDNA library of the sea urchin, *Hemicentrotus pulcherrimus*. The deduced amino acid sequence consisted of 523 residues which contained a 19-residue amino-terminal signal peptide and a 8-residue glycine-rich consensus sequences. Analysis of poly(A)$^+$RNA and/or total RNA from *H. pulcherrimus* testis, ovary, unfertilized eggs, and embryos by Northern blot revealed a 2.4 kb RNA.

INTRODUCTION

A sperm-activating peptide (SAP-I: GLy-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly), isolated from the egg jelly of sea urchins, *Hemicentrotus pulcherrimus* [13] and *Strongylocentrotus purpuratus* [3], increases sea urchin sperm respiration rate and motility. It induces a Na^+-dependent net proton efflux and raises the intracellular pH [10]. As the result SAP-I stimulates sperm energy metabolism which depends on the oxidation of endogenous phosphatidylcholine [8]. ATP synthesis by oxidative phosphorylation is a multistep membrane-located process that occurs in the inner membranes of mitochondria. F_0-F_1 ATP synthase (proton ATPase) in membranes of mitochondria synthesizes ATP coupled with an electrochemical gradient of protons generated by the electron transfer chain. The enzyme from many different sources have been studied extensively at the molecular biological level [2]. However, no molecular biological study has been made on the enzyme from spermatozoa of any kind of animals.

In this study, we screened a *H. pulcherrimus* testis cDNA library with oligonucleotide probes synthesized based on the amino acid sequence of peptide obtained from the protease V8 digest of wheat germ agglutinin (WGA)-binding protein of *H. pulcherrimus* spermatozoa and isolated a cDNA encoding the beta-subunit homologue of mitochondrial F_1-F_0 ATP synthase. Here, we report that the cDNA is 2259 bp long and an open reading frame predicts a protein 523 amino acids.

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[1] The nucleotide sequence data reported in this paper will appear in the DDBJ, GenBank and EMBL Nucleotide Sequence Databases with the following accession number D17361.

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MATERIALS AND METHODS

Cloning and sequencing of cDNA

A cDNA library (4.9×10^5 pfu) from poly(A)$^+$RNA isolated from growing testes of the sea urchin *H. pulcherrimus* was constructed in λ gt10 using the cDNA Synthesis System and the cDNA Cloning System λ gt10 (Amersham International plc., Amersham, UK). A 220 kDa WGA-binding protein was purified from *H. pulcherrimus* spermatozoa by affinity chromatography on a WGA-Sepharose 4B column as described previously [12], and digested by protease V8. The partial amino acid sequence of a peptide purified from the digest by preparative SDS-gel electrophoresis was determined to be V-S-S-*I-D-N-I-F-R*-V. The sequence indicated by italics was the same as the conserved sequence found in F_1-F_0 ATP synthase beta-subunit from various sources. Based on the sequence of the decapeptide, the mixed oligonucleotides (5′-GACACGGAAGATGTTGTCGATGCTGCTGAC-3′/5′-GACACGGAAGATGTTGTCGATAGAGGAGAC-3′) were synthesized and used to screen. Forty-six positive hybridizing clones were isolated from approximately 6×10^4 recombinants. Restriction endonuclease mapping of the inserts indicated that five different types of clones had been isolated. The insert of 2.3 kb from one member of the largest group in which fifteen clones belong was subcloned into the plasmid vector Bluescript II KS(+) (Stratagene, La Jolla, CA, USA) for further analysis. Serial deletion mutants of subclones were made according to Yanisch-Perron *et al* [16]. Nucleotide sequences were determined by the dideoxy chain termination method [11] using the Sequenase Kit (United States Biochemical Co., Cleaveland, OH, USA) and the 7-DEAZA Sequencing Kit (Takara Shuzo Co., Kyoto, Japan) analyzed on DANASIS software (Hitachi Software Engineering Co., Yokohama, Japan).

Northern blot analysis

Total RNA was prepared from testes, ovaryies, unfertilized eggs, and embryos of *H. pulcherrimus* by the LiCl method of Cathala *et al* [1]. Poly(A)$^+$RNA was prep ared by two passage of the total RNA over a column of oligo(dT)-cellulose (Pharmacia LKB Biotechnology, Uppsala, Sweden). Northern blot analysis was carried out as follows: 2–5 μg of poly(A)$^+$RNA or total RNA was denatured

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5'CGTGACCCCTGGAAGAATTTCACATCGCCATGTTTAGCAGGGTTGCAAAGACGAGTTTTTCGGCCGTAAGGGCTGCAAAATCACAATTT   89
   *                          M F S R V A K T S F S A V R A A K S Q F   20

TCACACTCATTATCACAACAGACGAGTAAAACATGGGTACCAGCAGCAACTTGTAGCAAAAGATCATATGCTGCTGAGGCAAAGACGTCG  179
 S H S L S Q Q T S K T W V P A A T C S K R S Y A A E A K T S   50

GCAGCCCCAGTTTCGGGTCAGATCGTAGCTGTCATTGGAGCTGTCGTCGACGTTCAGTTCGAGGATGACCTCCCACCCATTCTCAATGCC  269
 A A P V S G Q I V A V I G A V V D V Q F E D D L P P I L N A   80

TTGGAGGTTCAGGGAAGGACATCCAGGCTGGTGTTGGAAGTTGCACAGCATCTTGGTGAGAACACAGTCAGGACAATTGCCATGGACGGT  359
 L E V Q G R T S R L V L E V A Q H L G E N T V R T I A M D G   110

ACAGAAGGTCTGATCCGAGGCCAGAAGTGCGTTGACACTGGCTCCCCCATCAGCATCCCCGTCGGCCCCGAGACGCTGGGACGCATCATC  449
 T E G L I R G Q K C V D T G S P I S I P V G P E T L G R I I   140

AATGTCATTGGTGAACCCATTGACGAGAGAGGACCAATTGGAACAGACAGGAGATCAGCAATCCATGCAGAAGCTCCAGAGTTTGTAGAG  539
 N V I G E P I D E R G P I G T D R R S A I H A E A P E F V E   170

ATGAGTGTAAACCAGGAAATCCTTGTTACTGGAATCAAGGTTGTAGATCTACTCGCCCCATACGCCAAGGGAGGAAAGATTGGTCTGTTT  629
 M S V N Q E I L V T G I K V V D L L A P Y A K G G K I G L F   200

GGCGGTGCTGGTGTAGGAAAGACTGTACTCATCATGGAGCTGATTAACAACGTAGCCAAGGCCCACGGAGGTTACTCTGTGTTTGCCGGT  719
 G G A G V G K T V L I M E L I N N V A K A H G G Y S V F A G   230

GTAGGAGAGAGGACCCGTGAGGGTAACGATCTTTACCATGAGATGATTGAAGGAGGTGTCATCTCCCTCAAGGATGACACATCAAAGGTA  809
 V G E R T R E G N D L Y H E M I E G G V I S L K D D T S K V   260

GCGTTGGTGTACGGACAGATGAACGAGCCTCCCGGCGCCCGTGCCCGTGTCGCCTTGACCGGACTGACCGTTGCCGAATACTTCCGTGAC  899
 A L V Y G Q M N E P P G A R A R V A L T G L T V A E Y F R D   290

CAAGAGGGACAGGATGTGCTGCTCTTCATTGACAACATCTTCCGCTTCACACAGGCTGGATCAGAGGTATCTGCTCTGCTGGGACGTATC  989
 Q E G Q D V L L F I D N I F R F T Q A G S E V S A L L G R 1   320

CCATCTGCCGTAGGATACCAGCCAACCCTGGCCACTGACATGGGTACTATGCAGGAGCGTATTACCACCACCAAGAAGGGATCCATCACT 1079
 P S A V G Y Q P T L A T D M G T M Q E R I T T T K K G S I T   350

TCCGTACAGGCCATCTACGTGCCTGCTGACGATCTCACTGACCCTGCCCCTGCCACCACCTTCGCCCATTTGGACGCCACCACTGTGCTG 1169
 S V Q A I Y V P A D D L T D P A P A T T F A H L D A T T V L   380

TCCCGTGGTATCGCTGAGCTGGGTATCTACCCTGCTGTGGATCCTCTGGATTCCTCCTCCCGTATCATGGACCCCAACGTCGTCGGAGAG 1259
 S R G I A E L G I Y P A V D P L D S S S R I M D P N V V G E   410

CGTCACTACAGCATCGCTCGTGGAGTACAGAAAATCCTTCAGGACAACAAGACCCTGCAGGACATCATCGCCATCTTGGGTATGGACGAG 1349
 R H Y S I A R G V Q K I L Q D N K T L Q D I I A I L G M D E   440

TTGTCTGAGGACGACAAACTGACCGTGTCCCGAGCCAGGAAGATCCAGAGGTTCTTGTCCCAACCCTTCCAGGTTGCCGAGGTCTTCACC 1439
 L S E D D K L T V S R A R K I Q R F L S Q P F Q V A E V F T   470

GGCAGTCCAGGCAAGCTCGTCTCAATGGCGGAGACCATCGATGGATTCGAGTCCATTATCAAGGGCGAGTGCGACCATCTACCAGAGATT 1529
 G S P G K L V S M A E T I D G F E S I I K G E C D H L P E I   500

GCTTTCTACATGGTAGGCAACATTCAAGATGTCAAGGATAAGGCCGACAGGCTCGCAGAAGAACTATCATAAATTATCCCCCCTCTCCCA 1619
 A F Y M V G N I Q D V K D K A D R L A E E L S *               523

AACAATGAAGTTTAGAGCTGGCATGGCTACGGGTCAGAGACACCCCTCTTGATTGTTGTTATTCAGGGCTAGTTGTCTAACACTACCCGT 1709
GCCTGGGCCCAAAGAATTTATGTTCAGAGTTATAACTTATATCAAGATTGTTTTCTAAATTGTAATTGTGAAAAATTGAGAGCAAGGGAA 1799
TTCCAACCTAGCGTACTTTTGTCATATGAATCTGTCGTTTTCCTTCTTTTTTTTGCTTGTTATCCACCATAGATTGTAAATGCACAAACA 1889
GCTTGGCAAAGTTTGTAAATTTGATCATAACCAATTATCCCAATTTAAGGCAGTACCTTTAGCACATTGGTGTGTCACCGATGCCTGATT 1979
TCATGTTTATTGTCTGATCTGATCTTACAAGAAATTGGCCGATGTCCAAACATTTCCAATGTAGATATAGACATATATCTTCACTTGATT 2069
TCTGTGTAGAGCCGTTCACGTATGACAGATGATTGGCATTTATTTTGAATGGATGTTTTAGAGCTTTACTGAACCCAGTTGCGATTGTGA 2159
TTTCCTTGTGTGAACAGAATCGCAACTGGCCTTGAAAAAGAAAAACAAGTGTATTAAAAATTATTGGAAGGTTCAAGAACCAAAAAAAAA 2249
AAAAAAAAAA 3'                                                                               2259
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of the 2.3 kb insert. The shadowed box indicates predicted signal peptide sequence and the open box denotes glycine-rich consensus sequence. The amino acid sequence deisgnated by an underline is the same as partial sequence of the decapeptide used for synthesis of oligonucleotide probes. * denotes start or stop codon.

with 2.1 M formaldehyde, electrophoresed on a 1% agarose gel in the presence of 2.2 M formaldehyde, and transferred onto a Hybond-N-membrane. The RNA on the membrane was hybridized to the random-primed ECL labelled (Amersham International plc., Amersham, UK) or random-primed [α-^{32}P]dCTP-labelled 2.3 kb cDNA insert at 65°C for 18 hr. The membrane was washed with 0.5×SSC and 0.1% SDS at 65°C for 30 min. The size of the RNA was estimated using a 0.24–9.5 kb RNA Ladder (GIBCO BRL, Baithersburg, MD, USA) as a marker.

RESULTS AND DISCUSSION

The 2.3 kb insert contained DNA sequences encoding an open reading frame of 523 amino acids including I-D-N-I-F-R which is the same as the partial sequence of the peptide used for synthesis of oligonucleotide probes (Fig. 1). The deduced amino acid sequence suggests that the protein contains a 19-residue amito terminal signal peptide which has the potential to form amphipathic helix being characteristic of mitochondrial signal peptide sequence [5] and a 8-residue (residues 201-208) glycine-rich consensus sequence (G-X-X-X-X-G-K-T/S) found in the F_1-F_0 ATP synthase beta-subunit, adenylate kinase, p21 *ras* protein, and other nucleotide-binding proteins [14]. The deduced amino acid sequence has 68% homology with those of chloroplast F_1-F_0 ATP synthase beta-subunits and 85% with those of mitochondrial F_1-F_0 ATP synthase beta-subunits from various

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                                10        20        30        40        50        60
Spermatozoa (sea urchin)   MFSRVAKTSFSAVRAAKSQFSHSLSQQTSKTWVPAATCSKRSYAAEAKTSA--APVSGQIVAVIGAVVDV
Mitochondria (human)    MLGFVG...AAPA.GALRRLTPSASLPPA.LLLRAA.T.VHPV.D...QTSP.PKAGAAT.R..........
Mitochondria (rat)      MLSLVG...SA.A.GALRGLNPLAALPQAHLLLRTA..GVHPA.D...QSSAAPKAGTAT............
Chloroplast (potato)                                                MRINPTTSGS.VS.VE--KKNL.R..KI..P.L..
Chloroplast (spinach)                                               MRINPTTSDPGVS.LE--KKNL.R.AQI..P.LN.

      70        80              90        100       110       120       130       140       150
QF-EDDLPPILNALEVQGR----TS---RLVLEVAQHLGENTVRTIAMDGTEGLIRGQKCVDTGSPISIPVGPETLGRIINVIGEPIDER
..-DEG.............----ET---.............S.............V....VL.S.A..K...........M..........
..-DEG.............----E.---.............S.............V....VL.S.A..K...........M..........
A.PPGKM.N.Y...V....----GNEQTNVTC..Q.L..N.R..AV..SD.D..M..MEVI...A...V...GS.....F..L.Q.V.NL
A.PPGKM.N.Y...I.K..DTAGQPM--NVTC..Q.L..N.R..AV..SA.D..T..MEVI...A.L.V...GP.....F..L...V.NL

      160       170       180       190       200       210       220       230       240
GPIGTDRRSAIHAEAPEFVEMSVNQEILVTGIKVVDLLAPYAKGGKIGLFGGAGVGKTVLIMELINNVAKAHGGYSVFAGVGERTREGND
...K.KQFAP........M....E.....................................................................
...K.KQFAP........I....E.....................................................................
..VD.NTT.P..RS..A.IQLDTKLS.FE............RR.......................I.......V...G...........
R.VD.RTT.P..RS..A.TQLDTKLS.FE......N.....RR.......................I.......V...G...........

      250       260       270       280       290       300       310       320       330
LYHEMIEGGVISLKD-DTSKVALVYGQMNEPPGARARVALTGLTVAEYFRDQEGQDVLLFIDNIFRFTQAGSEVSALLGRIPSAVGYQPT
.......S...N...-A.....................................................................
.......S...N...-A.....................................................................
..L..K.S...NEENIPE.........-.......M..G..A..M......VNE.............V............M.........
..M..K.S...NEQNIAE.................M..G..A..M......VNE.............V............M.........

      340       350       360       370       380       390       400       410       420
LATDMGTMQERITTTKKGSITSVQAIYVPADDLTDPAPATTFAHLDATTVLSRGIAELGIYPAVDPLDSSSRIMDPNVVGERHYSIARGV
.....................................................A.............T.......I..SE..DV....
.....................................................A.............T.......I..SE..DV....
.S.E..YL.....S..E.....I..V.........................L.AK...........T.TMLQ.RI...E..ET....
.S.E..SL.....S..E.....I..V.........................L.AK...........T.TMLQ.RI...E..E..QR.

      430       440       450       460       470       480       490       500       510
QKILQDNKTLQDIIAILGMDELSEDDKLTVSRARKIQRFLSQPFQVAEVFTGSPGKLVSMAETIDGFESIIKGECDHLPEIAFYMVGNIQ
......Y.S.............E.........................HM....PLK...K..QQ.LA..Y.....Q......P.E
......Y.S.............E.........................HM....PLK...K..QQ.LA.DY....Q......P.E
KQT..RY.E.........L.....E.R...A.....E.......F...........Y.GL....R..QL.LS..L.G...Q...L....D
KET..RY.E.........L.....E.R...A.....E.......F...........Y.GL....R..QL.LS..L.S...Q...L....D

      520                 Homology
DVKDKADRLAEELS              100%
EAVA...K....H.S              85%
EAVA...K....HGS              85%
EATA..MN.KT                  68%
EATA..MN.EM.SKLKK            68%
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FIG. 2. Comparison of deduced amino acid sequence of the sea urchin homologue and mitochondrial (human [9], rat [4]) and chloroplast (potato [7], spinach [17]) F_1-F_0 ATP synthase beta-subunits. Dots indicate the same amino acid residues as sea urchin homologue and positions where gap have been introduced for maximum homology are indicated by a dash.

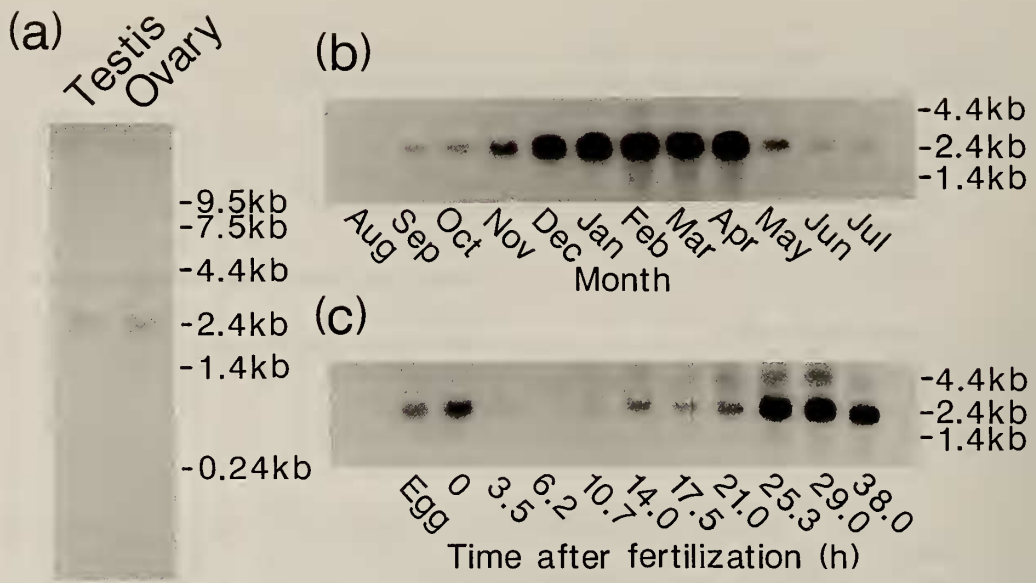

FIG. 3. Analysis of RNA prepared from *H. pulcherrimus* ovaies, testis, unfertilized eggs and embryos by Northern blot hybridization. (a): poly(A)$^+$RNA (2 μg) prepared from ovaries and testis samples collected in March, detected by ECL; (b): total RNA (5 μg) from the testis samples collected throughout the year, detected by autoradiography; (c); total RNA (5 μg) from unfertilized eggs and embryos cultured at 20°C, detected by autoradiography.

sources (Fig. 2) [4, 7, 9, 17]. This suggests that the cDNA clone isolated from the *H. pulcherrimus* testis cDNA library codes for the beta-subunit of mitochondrial F_1-F_0 ATP synthase and the primary strucutres of the beta-subunits are highly conserved in very different species.

Norhtern blot analysis using the 2.3 kb insert as a probe indicated that the mRNA of 2.4 kb presents both in the ovary and testis of the sea urchin (Fig. 3a). In previous study, we demonstrated that *H. pulcherrimus* spermatozoa contained a large amount of membrane-bound guanylate cyclase and creatine kinase and the activities of both enzymes increased during the testis development [6]. As shown in Figure 3b, the mRNA encoding F_1-F_0 ATP synthase beta-subunit began to the accumulated in the testis collected in November when spermatogenic cells appeared along the wall of testicular lobes, suggesting that F_1-F_0 ATP synthase is also synthesized in the testis with formation of mature spermatozoa. The mRNA was also identified in unfertilized eggs and developing embryos, while the signal of hybridizing RNA from the unfertillized eggs was weaker than that from the developing embryos (Fig. 3c). This may be due to imcomplete polyadenylation of the stored mRNA in unfertilized eggs [15]. Additional polyadenylation reaction appears to begin rapidly upon fertilization (Fig. 3c). The mRNA was not appreciably detected in the embryos during early cleavage stage and became detectable in the embryos of the gastrula stage (Fig. 3c).

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REFERENCES

1 Cathala G, Savouret J-F, Mendez B, West BL, Karin M, Martial JA, Baxter JD (1983) DNA 2: 329–335
2 Futai M, Noumi T, Maeda M (1989) Am Rev Biochem 58: 111–136
3 Garbers DL, Watkins HD, Hansbrough JR, Smith AC, Misono KS (1982) J Biol Chem 257: 2734–2737
4 Garboczi DN, Fox AH, Gerring SL, Pedersen PL (1988) Biochemistry 27: 553–560
5 Hawlistschek G, Schneider H, Schmidt B, Tropschung M, Hartl F-U, Neupert W (1988) Cell 53: 795–806
6 Harumi T, Kurita M, Suzuki N (1992) Develop Growth Differ 34: 151–162
7 Kobayashi K, Nakamura K, Asahi T (1987) Nucleic Acids Res 15: 7177–7177
8 Mita M, Ueta N, Harumi T, Suzuki N (1990) Biochim Biophys Acta 1035: 175–181
9 Ohta S, Tomura H, Matsuda K, Kagawa Y (1988) J Biol Chem 263: 11257–11262
10 Repaske DR, Garbers DL (1983) J Biol Chem 258: 6025–6029
11 Sanger R, Nicklen S, Coulson AR (1977) Proc Natl Acad Sci USA 74: 5463–5467
12 Sendai Y, Aketa K (1991) Dev Growth Differ 33: 101–109
13 Suzuki N, Nomura K, Ohtake H, Isaka S (1981) Biochem Biophis Res Commun 99: 1238–1244
14 Walker JE, Saraste M, Runswick MJ, Gay NJ (1982) EMBO J 1: 945–951
15 Wilt FH (1973) Proc Natl Acad Sci USA 70: 2345–2349
16 Yanisch-Perron C, Vieira J, Messing J (1985) Gene 33: 103–119
17 Zurawski G, Bottomley W, Whitfeld PR (1982) Proc Natl Acad Sci USA 79: 6260–6264